

Table 7 (Continued)

	<u>Test compound</u>	<u>Tumor Cell Line Type</u>	<u>Cell Line Designation</u>
	PRO866	NSCL	HOP-62
	PRO866	NSCL	HOP-92
5	PRO866	Colon	KM12
	PRO866	CNS	SF-295
	PRO866	Ovarian	IGROV1
	PRO866	Breast	MDA-MB-435
	PRO866	Melanoma	LOX IMVI
10	PRO320	Leukemia	CCRF-CEM; RPMI-8226
	PRO320	NSCL	HOP62; NCI H322M
	PRO320	Colon	HCT-116
	PRO320	Renal	SN12C
	PRO320	Breast	MDA-N
	PRO320	Ovarian	OVCAR-3
15	PRO320	Melanoma	MALME-3M

* cytotoxic

The results of these assays demonstrate that the positive testing PRO polypeptides are useful for inhibiting neoplastic growth in a number of different tumor cell types and may be used therapeutically therefor. Antibodies against these PRO polypeptides are useful for affinity purification of these useful polypeptides. Nucleic acids encoding these PRO polypeptides are useful for the recombinant preparation of these polypeptides.

EXAMPLE 114: Gene Amplification in Tumors

This example shows that certain PRO polypeptide-encoding genes are amplified in the genome of certain human lung, colon and/or breast cancers and/or cell lines. Amplification is associated with overexpression of the gene product, indicating that the polypeptides are useful targets for therapeutic intervention in certain cancers such as colon, lung, breast and other cancers and diagnostic determination of the presence of those cancers. Therapeutic agents may take the form of antagonists of the PRO polypeptide, for example, murine-human chimeric, humanized or human antibodies against a PRO polypeptide.

The starting material for the screen was genomic DNA isolated from a variety cancers. The DNA is quantitated precisely, *e.g.*, fluorometrically. As a negative control, DNA was isolated from the cells of ten normal healthy individuals which was pooled and used as assay controls for the gene copy in healthy individuals (not shown). The 5' nuclease assay (for example, TaqMan™) and real-time quantitative PCR (for example, ABI Prizm 7700 Sequence Detection System™ (Perkin Elmer, Applied Biosystems Division, Foster City, CA)), were used to find genes potentially amplified in certain cancers. The results were used to determine whether the DNA encoding the PRO polypeptide is over-represented in any of the primary lung or colon cancers or cancer cell lines or breast cancer cell lines that were screened. The primary lung cancers were obtained from individuals with tumors of the type and stage as indicated in Table 8. An explanation of the abbreviations used for the designation of the primary tumors listed in Table 8 and the primary tumors and cell lines referred to throughout this example are given below.

The results of the TaqMan™ are reported in delta (Δ) Ct units. One unit corresponds to 1 PCR cycle or approximately a 2-fold amplification relative to normal, two units corresponds to 4-fold, 3 units to 8-fold amplification and so on. Quantitation was obtained using primers and a TaqMan™ fluorescent probe derived

from the PRO polypeptide-encoding gene. Regions of the PRO polypeptide-encoding gene which are most likely to contain unique nucleic acid sequences and which are least likely to have spliced out introns are preferred for the primer and probe derivation, *e.g.*, 3'-untranslated regions. The sequences for the primers and probes (forward, reverse and probe) used for the PRO polypeptide gene amplification analysis were as follows:

PRO853 (DNA48227-1350)

5 48227.tm.fl
5'-GGCACTTCATGGTCCTTGAAA-3' (SEQ ID NO:539)

48227.tm.p1
5'-CGGATGTGTGTGAGGCCATGCC-3' (SEQ ID NO:540)
48227.tm.r1

10 5'-GAAAGTAACCACGGAGGTCAAGAT-3' (SEQ ID NO:541)

PRO1017 (DNA56112-1379):

56112.tm.fl
5'-CCTCCTCCGAGACTGAAAGCT-3' (SEQ ID NO:542)

15 56112.tm.p1
5'-TCGCGTTGCTTTTCTCGCGTG-3' (SEQ ID NO:543)
56112.tm.r1
5'-GCGTGCGTCAGGTTCCA-3' (SEQ ID NO:544)

20 PRO213-1 (DNA30943-1163-1):

30943.tm.f3:
5'-CGTTCGTGCAGCGTGTGTA-3' (SEQ ID NO:545)

30943.tm.p3:
5'-CTTCCTCACCACCTGCGACGGG-3' (SEQ ID NO:546)

25 30943.tm.r3:
5'-GGTAGGCGGTCCTATAGATGGTT-3' (SEQ ID NO:547)

30943.tm.fl:
5'-AGATGTGGATGAATGCAGTGCTA-3' (SEQ ID NO:548)

30943.tm.p1:
30 5'-ATCAACACCGCCGGCAGTTACTGG-3' (SEQ ID NO:549)

30943.tm.r1:
5'-ACAGAGTGTACCGTCTGCAGACA-3' (SEQ ID NO:550)

30943.3trn-5:
5'-AGCCTCCTGGTGCACTCCT-3' (SEQ ID NO:551)

35 30943.3trn-probe:
5'-CGACTCCCTGAGCGAGCAGATTTCC-3' (SEQ ID NO:552)

30943.3trn-3:

36343.25613

5'-GCTGGGCAGTCACGAGTCTT-3' (SEQ ID NO:553)

PRO237 (DNA34353-1428):

34353.tm.f:

5'-AATCCTCCATCTCAGATCTTCCAG-3' (SEQ ID NO:554)

5 34353.tm.p:

5'-CCTCAGCGGTAACAGCCGGCC-3' (SEQ ID NO:555)

34353.tm.r:

5'-TGGGCCAAGGGCTGC-3' (SEQ ID NO:556)

10 PRO324 (DNA36343-1310):

36343.tmf1:

5'-TGGTGGATAACCAACAAGATGG-3' (SEQ ID NO:557)

36343.tmp1:

5'-GAGTCTGCATCCACACCACTCTTAAAGTTCTCAA-3' (SEQ ID NO:558)

15 36343.tmr1:

5'-CAGGTGCTCTTTTCAGTCATGTTT-3' (SEQ ID NO:559)

PRO351 (DNA40571-1315):

40571.tm.fl:

20 5'-TGGCCATTCTCAGGACAAGAG-3' (SEQ ID NO:560)

40571.tm.p1:

5'-CAGTAATGCCATTTGCCTGCCTGCAT-3' (SEQ ID NO:561)

40571.tm.r1:

5'-TGCCTGGAATCACATGACA-3' (SEQ ID NO:562)

25

PRO362 (DNA45416-1251):

45416.tm.fl:

5'-TGTGGCACAGACCCAATCCT-3' (SEQ ID NO:563)

45416.tm.p1:

30 5'-GACCCTGAAGGCCTCCGGCCT-3' (SEQ ID NO:564)

45416.tm.r1:

5'-GAGAGAGGGAAGGCAGCTATGTC-3' (SEQ ID NO:565)

PRO615 (DNA48304-1323):

35 48304.tm.fl:

5'-CAGCCCCTCTCTTTCACCTGT-3' (SEQ ID NO:566)

48304.tm.p1:

5'-CCATCCTGTGCAGCTGACACACAGC-3' (SEQ ID NO:567)